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Title: BUFFERED FORMULATIONS FOR CONCENTRATING ANTIBODIES

Atty. Dkt. PW 0280724 M# | 2002-30-0019P Client Ref

Date: June 21, 2002

including:

1. Specification: 38 pages 2. ☐ Specification in non-English language 3. ☒ Drawings: 7 sheet(s)
4. The invention ☐ was ☒ was not made by, or under a contract with, an agency of the U.S. Government.
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8. This application is made by the following named inventor(s) **(Double check instructions for accuracy.):**

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BUFFERED FORMULATIONS FOR CONCENTRATING ANTIBODIES

FIELD OF THE INVENTION

The present invention relates to buffered antibody preparations that provide for efficient concentration of the antibodies by a membrane filtration process; to a process for concentrating antibodies in which such a preparation is subjected to a membrane filtration process; to a concentrated antibody preparation produced by the process; and to using the concentrated antibody preparations produced by the process to prepare pharmaceutical antibody formulations useful for human therapy.

BACKGROUND

Immunoglobulin G (IgG) preparations have been purified for use in human therapy since the 1940s. At present, human therapeutic immunoglobulin products are marketed commercially as 16% (w/v) (160 mg/ml) solutions for intramuscular administration, e.g., for hepatitis A prophylaxis, and as 5% (w/v) (50 mg/ml) solutions for intravenous administration, e.g., for treatment of primary immunodeficiencies, infections, and autoimmune diseases. See column 1 of U.S. Patent No. 6,252,055, the contents of which are incorporated herein in their entirety.

Efforts to develop therapeutic monoclonal antibodies (MAbs) that act like "magic bullets" by targeting disease-causing antigens attained success in the late 1990s. In 1997, the FDA approved Rituxan, a chimeric anti-CD20 antibody from IDEC Pharmaceuticals Corp. and Genentech, Inc., for the treatment of non-Hodgkin's lymphoma. This was the first MAb to be approved by the FDA. Other therapeutic antibodies have since been approved by the FDA for various indications, such as Herceptin (Genentech, Inc.) for the treatment of breast cancer, Synagis (Medimmune, Inc.) for treating Respiratory Syncytial

virus infections in children, and Remicade (Centocor, Inc.) for treating Crohn's disease. (See H. Iyer et al., BioPharm, January, 2002, page 14).

Many therapeutic MAbs are currently undergoing clinical testing for FDA approval. One example is IDEC-114, an anti-CD80 MAb for treating autoimmune diseases and preventing organ transplant rejection that is described in U.S. Patent No. 6,113,898, the contents of which are incorporated herein in their entirety. Another is IDEC-131, an anti-gp39 MAb that is also useful for treating autoimmune diseases, as described in U.S. Patent No. 6,001,358, the contents of which are incorporated herein in their entirety. A third example is IDEC-151, an anti-CD4 MAb that is useful for T cell depletion therapy, e.g., to provide immunosuppression, as described in U.S. Patent No. 6,136,310, the contents of which are incorporated herein in their entirety. Another therapeutic MAb being evaluated for therapeutic use is IDEC-152, an anti-CD23 antibody that inhibits IL-4-induced IgE production by B cells and is useful for treating IgE-mediated pathologies such as atopic dermatitis, allergic rhinitis, and asthma, as described in U.S. Patent No. 6,011,138, the contents of which are incorporated herein in their entirety.

Effective treatment with therapeutic MAbs typically requires repeated administration of doses of a therapeutic preparation of MAbs that are concentrated to 100 mg/ml or greater. Therapeutic MAbs are commonly administered parenterally, by intravenous, intramuscular, or intraperitoneal delivery. The patient is frequently hospitalized during administration, because of the large volume of MAb solution that must be administered, and to permit observation of the patient's response to treatment. There is considerable interest in developing efficient methods for preparing highly concentrated preparations of therapeutic MAbs, in order to reduce the volume of solution that contains the required dosage, and so reduce the infusion time required for administration.

There is also considerable interest in developing efficient methods for preparing highly concentrated preparations of therapeutic MAbs that are suitable for subcutaneous administration, which have the advantage that they can be self-administered. Since the volume of a dose that can be administered by the subcutaneous route is relatively small (about 1 ml), the concentration of MAbs in a preparation of therapeutic MAbs that is to be administered effectively by the subcutaneous route should be in the range of 100 to 200 mg/ml. In general, it is desirable that the concentration of MAbs in a preparation of therapeutic MAbs be between 100 and 300 mg/ml (see column 4 of U.S. Patent No. 6,252,055).

Diverse methods for preparing and purifying therapeutic MAbs have been developed. These generally have in common a final step of concentration by ultrafiltration that produces the concentrated antibody preparation that is formulated into the pharmaceutical preparation that is to be administered. Ultrafiltration of MAbs is carried out by filtering the antibody solution under pressure through a membrane filter with pores that retain the MAbs while allowing smaller molecules to pass through. Commonly used methods for ultrafiltration are discussed below, in the Detailed Description of the Invention.

In preparing highly concentrated antibody preparations for pharmaceutical formulation, it is important to prevent the formation of antibody aggregates and to remove any aggregated antibodies that form in solutions that are used to prepare pharmaceutical formulations, because antibody aggregates can cause a number of adverse side-effects if they are present in a pharmaceutical formulation that is administered to a patient. See column 1 of U.S. Patent No. 5,608,038. The contents of U.S. Patent No. 5,608,038 are incorporated herein in their entirety. Aggregates are readily removed from a concentrated antibody solution by microfiltration.

It is also important to find conditions that reduce the viscosity of the antibody preparation, in order to increase the rate of filtration, maximize recovery (by reducing the sticking of material to tubing, plasticware, etc.), improve the ease of handling and the accuracy of concentration determinations, and to provide a pharmaceutical formulation that can be administered quickly to people with narrow veins, such as children. See column 2 of U.S. Patent No. 5,608,038.

U.S. Patent No. 5,608,038 describes a stable antibody preparation that is concentrated to 135-175 mg/ml, has acceptably low viscosity, and comprises a stabilizing agent; and a method for making such an antibody preparation by adjusting the concentration of saccharides such as glucose or sucrose in the preparation to be sufficient to give osmolarity of between 250 and 600 mOs/l and to stabilise the antibodies. The patent teaches that the saccharide concentration in the antibody preparation is preferably in the range of from 30 to 50 mg/ml (see col. 2).

U.S. Patent No. 6,252,055 describes a method for making a preparation of MAbs concentrated to 100 mg/ml or greater that has acceptably low viscosity and is free of stabilizing excipients such as glycine and/or maltose, wherein the antibodies are combined with a carrier such as physiologic saline, phosphate buffered saline, glucose and buffered saline, citrate buffered saline, citric acid/sodium citrate buffer, maleate buffer, succinate buffer, acetate buffer, for example sodium acetate/acetic acid buffer, or phosphate buffer (col. 3, lines 45-58), with such buffers preferably being used at a concentration of 50-100 mM, with the pH adjusted to be different from the isoelectric point of the antibody (col. 4). Patent No. 6,252,055 teaches that a marked increase in viscosity can occur when antibody concentration is raised above 190 mg/ml (col. 6); and that the level of aggregation (as measured by size exclusion chromatography) appears to be unrelated to this concentration-dependent change in viscosity (col. 7 and col. 8). The patent also

discloses that a MAb can be concentrated to greater that 150 mg/ml by tangential flow filtration at 2-2.5 bar without significant precipitation when the retentate is circulated at a flow rate of 250 ml/min (col. 10).

U.S. Patent No. 6,171,586 discloses a method for stabilizing an antibody in an aqueous pharmaceutical formulation by combining a therapeutically effective amount of the antibody, a buffer, a surfactant, and a polyol.

U.S. Patent Application No. US 2002/0045571 discloses a formulation comprising a protein in an amount of at least 80 mg/ml and a salt or buffer in an amount of at least 50 mM, and having kinematic viscosity of about 50 centistokes or less.

Notwithstanding what has been previously described, there still exists a need in the art for improved methods for preparing highly concentrated antibody preparations free of excipients, with lowered viscosity and reduced aggregation, that can be used in pharmaceutical formulations.

SUMMARY AND OBJECTS OF THE INVENTION

The present invention relates to a buffered antibody preparation that is particularly suitable for being subjected to a membrane filtration process for further concentration of the antibodies; to a process for concentrating antibodies comprising subjecting such a preparation to membrane filtration; to a concentrated antibody preparation obtained by such a membrane filtration process; and to using concentrated antibody preparations obtained by the process in preparing pharmaceutical antibody formulations useful for therapy.

It is an object of the invention to provide a composition of antibodies that is suitable for subjecting to further concentration by membrane filtration and comprises

histidine or acetate buffer at a concentration in the range of from 5 to 40 mM; preferably in the range of from 15 to 30 mM, and more preferably in the range of from 20 to 25 mM.

Another object of the invention is to provide a composition of antibodies that is suitable for subjecting to further concentration by membrane filtration and comprises histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM, and has pH in the range of from 4.5 to 7.0; preferably in the range of from 5.0 to 6.5; and more preferably in the range of 5.5 to 6.0.

It is also an object of the invention to provide an antibody composition suitable for subjecting to further concentration by membrane filtration that comprises IgG antibodies and histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM.

An additional object of the invention is to provide an antibody composition suitable for subjecting to further concentration by membrane filtration that comprises monoclonal antibodies and histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM.

A preferred embodiment of the invention is the above-described composition of monoclonal antibodies for which the concentration of monoclonal antibodies is in the range of from 25 to 350 mg/ml; preferably in the range of from 50 to 250 mg/ml; and more preferably, is at least 100 mg/ml.

An additional object of the invention is to provide an antibody composition suitable for subjecting to further concentration by membrane filtration that comprises primatized monoclonal antibodies and histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM.

Another object of the invention is to provide an antibody composition suitable for subjecting to further concentration by membrane filtration that comprises histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM, and also comprises

monoclonal antibodies selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies.

An additional object of the invention is to provide an antibody composition suitable for subjecting to further concentration by membrane filtration that comprises histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM, and further comprises monoclonal antibodies selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies.

It is another object of the invention to provide a method for producing a concentrated antibody preparation comprising the steps of (a) providing an initial antibody preparation comprising histidine or acetate buffer; and (b) subjecting the initial antibody preparation to membrane filtration that removes water and buffer but not antibodies from the antibody preparation, to produce a concentrated antibody preparation comprising histidine or acetate buffer at a concentration in the range of from 5 to 40 mM; preferably in the range of 15 to 30 mM, and more preferably in the range of 20 to 25 mM.

Another object of the invention is to provide the above-described method for producing a concentrated antibody preparation having pH in the range of from 4.5 to 7.0; preferably in the range of from 5.0 to 6.5; and more preferably in the range of 5.5 to 6.0.

It is also an object of the invention is to provide the above-described method for producing a concentrated antibody preparation that comprises IgG antibodies.

An additional object of the invention is to provide the above-described method for producing a concentrated antibody preparation that comprises monoclonal antibodies.

Another object of the invention is to provide the above-described method for producing a concentrated antibody preparation that comprises monoclonal antibodies present at a concentration in the range of from 25 to 350 mg/ml; preferably in the range of from 50 to 250 mg/ml; and more preferably, at least 100 mg/ml.

It is an object of the invention is to provide the above-described method for producing a concentrated antibody preparation that comprises primatized monoclonal antibodies.

It is an additional object of the invention to provide the above-described method for producing a concentrated antibody preparation that comprises monoclonal antibodies selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies.

Another object of the invention is to provide the above-described method for producing a concentrated antibody preparation that comprises monoclonal antibodies selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies.

A preferred method for concentrating antibodies by membrane filtration according to the present invention is ultrafiltration by tangential flow filtration.

Various methods have been developed for concentrating antibodies in an antibody preparation by subjecting it to a process of membrane filtration that removes solvent and small molecules water but not antibodies from the antibody preparation. Such methods are carried out using both normal flow filtration and tangential flow filtration. The present invention provides an improvement over previously described methods for concentrating a buffered solution of antibodies by membrane filtration, the improvement being that the antibody preparation that is subjected to membrane filtration is one that comprises histidine or acetate buffer at a concentration in the range of 5 to 40 mM; preferably in the range of 15 to 30 mM, and more preferably in the range of 20 to 25 mM.

It is another object of the present invention to provide a method for producing a pharmaceutical composition comprising antibodies as the active ingredient, comprising the steps of (a) providing an initial antibody preparation comprising histidine or acetate buffer

a concentration in the range of from 5 mM to 40 mM; preferably in the range of 15 to 30 mM, and more preferably in the range of 20 to 25 mM; (b) subjecting the initial antibody preparation to membrane filtration that removes water and buffer but not antibodies from the antibody preparation, thereby producing an antibody preparation having a higher concentration of antibodies than the initial antibody preparation; and (c) combining antibodies of the concentrated antibody preparation of step (b) with one or more pharmaceutically acceptable carriers to produce a pharmaceutical composition.

Another object of the present invention is to provide the method for producing a pharmaceutical composition comprising antibodies as the active ingredient as described above, wherein the pH of the initial antibody solution is in the range of from 4.5 to 7.0; preferably in the range of from 5.0 to 6.5; and more preferably in the range of 5.5 to 6.0.

Another object of the present invention is to provide the method for producing a pharmaceutical composition comprising antibodies as the active ingredient as described above, wherein the antibodies are IgG antibodies.

Another object of the present invention is to provide the method for producing a pharmaceutical composition comprising antibodies as the active ingredient as described above, wherein the antibodies are monoclonal antibodies.

Another object of the present invention is to provide the method for producing a pharmaceutical composition comprising antibodies as the active ingredient as described above, wherein the antibodies are monoclonal antibodies present at a concentration in the range of from 25 to 350 mg/ml; preferably in the range of from 50 to 250 mg/ml; and more preferably, at least 100 mg/ml.

Another object of the present invention is to provide the method for producing a pharmaceutical composition comprising antibodies as the active ingredient as described above, wherein the antibodies are primatized monoclonal antibodies.

Another object of the present invention is to provide the method for producing a pharmaceutical composition comprising antibodies as the active ingredient as described above, wherein the antibodies are monoclonal antibodies selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies.

Another object of the present invention is to provide the method for producing a pharmaceutical composition comprising antibodies as the active ingredient as described above, wherein the antibodies are monoclonal antibodies selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies.

It is also an object of the present invention to provide an improved method of therapy that includes the administration of a pharmaceutical composition comprising an antibody, with the improvement comprising administering a pharmaceutical composition that comprises a concentrated antibody preparation comprising histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM, and further comprises a therapeutically effective dose of a therapeutic antibody.

It is an object of the present invention that the above-described improved method of therapy comprises administering a therapeutically effective dose of therapeutic antibody to a patient suffering from a disease selected from the group consisting of cancer, allergic disorders, autoimmune diseases, and lymphoma.

It is an object of the present invention that the above-described improved method of therapy also comprises administering a therapeutically effective dose of therapeutic IgG antibodies.

Another object of the present invention is that the above-described improved method of therapy comprises administering a therapeutically effective dose of therapeutic monoclonal antibodies.

Another object of the present invention is that the above-described improved method of therapy comprises administering a therapeutically effective dose of therapeutic primatized monoclonal antibodies.

Another object of the present invention is that the above-described improved method of therapy comprises administering a therapeutically effective dose of therapeutic monoclonal antibodies selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies.

It is also an object of the present invention that the above-described improved method of therapy comprises administering a therapeutically effective dose of therapeutic monoclonal antibodies selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies.

An additional object of the present invention is to provide a kit that is useful for the treatment of a mammal suffering from, or predisposed to, a disorder. Inside the kit is at least one container having a pharmaceutical composition prepared from a concentrated antibody preparation comprising a therapeutically effective dose of a therapeutic antibody useful for treating said disorder, and also comprising histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM, according to the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 schematically depicts direct flow filtration (DFF). The feed, i.e., the solution to be filtered, is forced directly toward the membrane as shown in Figure 1. The smaller molecules pass through the pores as the filtrate while the larger antibodies are retained by the membrane. The molecules larger than the pores are shown aggregating at the membrane surface and forming a gel.

Figure 2 is a graph showing that the flux rate during DFF decreases rapidly as filtration proceeds, because the antibodies aggregate at the membrane surface and form a gel that blocks the flow of the smaller molecules through the pores.

Figure 3 schematically depicts tangential flow filtration (TFF). Once the feed is introduced into the system, the solution circulates so that the sample flows across the surface of the membrane while pressure in the solution forces smaller molecules in the solution through the pores of the membrane as filtrate. The solution and antibody molecules that remain between the membranes form the retentate.

Figure 4 is a graph showing that the flux rate during TFF decreases gradually as filtration proceeds.

Figure 5 is a graph that shows the dependence of filtration flow rate on antibody concentration for solutions containing three different buffers at pH 5.5 and pH 6.0. From the data plotted in the graph, it can be seen that filtration flow rate at a wide range of antibody concentrations is markedly greater with histidine and acetate buffers than with citrate buffer. There do not appear to be significant differences between flow rates achieved at pH 5.5 and pH 6.0.

Figure 6 is a graph that shows the change in OD320, a measure of turbidity, with increases in antibody concentration over the course of TFF, for solutions containing three different buffers at pH 5.5 and pH 6.0. It can be seen from the graph that the formulation containing citrate buffer had the highest turbidity, there was intermediate turbidity in the

tate-containing formulation, and the formulation containing histidine had the lowest turbidity.

Figure 7 is a bar graph representing the kinematic viscosities of solutions of IDEC-114 formulated at 135 mg/ml with different buffers at pH 5.5 and 6.0. The citrate-containing formulations had significantly higher viscosities than the others. Viscosities of formulations at pH 6.0 also are consistently higher than those at pH 5.5.

DETAILED DESCRIPTION OF THE INVENTION

Antibody therapeutics can be used successfully to treat a number of oncology- and immune system-related indications; however, large dosages of an antibody drug are often required if the drug is to be therapeutically effective. In order to deliver a therapeutically effective dosage of an antibody to a patient by intravenous or subcutaneous routes, the concentration of the antibody preparation usually must be high, a requirement that frequently creates difficulties, both in preparing the drug and in maintaining it in stable form.

The present invention is directed to providing compositions and methods that permit the production of highly concentrated, stable antibody preparations of relatively low viscosity that are substantially free of aggregates and are suitable for use in a pharmaceutical formulation.

In one embodiment, the present invention is a method for producing a concentrated antibody preparation. The steps of the method are:

(a) providing an initial antibody preparation consisting essentially of an aqueous solution of antibodies and histidine or acetate buffer at a concentration in the range of from 5 to 40 mM; preferably in the range of from 15 to 30 mM, and more preferably in the range of from 20 to 25 mM; and

(b) subjecting the initial antibody preparation to membrane filtration that removes water and buffer but not the antibodies from the antibody preparation, thereby producing an antibody preparation having a higher concentration of antibodies than the initial antibody preparation.

The present invention springs from the unexpected observation that low concentrations of acetate or histidine buffer (5-40 mM) are able to stabilize an antibody preparation during concentration by membrane filtration, lowering the viscosity of the antibody solution, and suppressing aggregation, to an extent that equals or surpasses the stabilizing effects that have been achieved using other, more complex formulations described in the art. As described below, preparations of histidine- and acetate-formulated antibodies outperform citrate-formulated antibody preparations at similar pH ranges, in terms of both operational efficiency and product stability. Histidine- and acetate-formulated antibody preparations are shown to have lower viscosity and less aggregation in comparison to citrate-formulated preparations.

The present invention also provides and includes compositions of concentrated antibodies that are prepared by practicing the above-described method, as well as the pharmaceutical formulations comprising concentrated antibody preparations that are produced according to the present invention.

The invention also provides an antibody preparation consisting essentially of an aqueous solution of antibodies and histidine or acetate buffer at a concentration in the range of from 5 to 40 mM, and methods for making and using such compositions, having pH in the range of from 4.5 to 7.0; preferably in the range of from 5.0 to 6.5; and more preferably in the range of 5.5 to 6.0. Such solutions can be made by common methods well-known to those in the art. Preferably, the acetate buffer is Na-acetate, and the histidine buffer is histidine HCl; however, the invention can be practiced successfully by

employing counterions other than Na^+ and Cl^- when adjusting the pH to the above-stated values.

The present invention may be used for concentrating preparations of any type of antibody; for example, it may be used for concentrating preparations of immunoglobulins of all classes, i.e. IgM, IgG, IgA, IgE and IgD, and also may be used for concentrating a preparation of Fab fragments, or a preparation of bispecific antibodies. The invention is preferably applied to a preparation of immunoglobulins of the class IgG, which includes the sub-classes IgG_1, IgG_2, IgG_3 and IgG_4. The invention is more preferably applied to a preparation of immunoglobulins of the class IgG_4 and IgG_1, most preferably IgG_1.

The present invention is particularly useful for producing highly concentrated preparations of monoclonal antibodies, preferably at a concentration in the range of from 25 to 350 mg/ml; more preferably in the range of from 50 to 250 mg/ml; and even more preferably, at a concentration of at least 100 mg/ml.

The invention may also be used for producing highly concentrated preparations of recombinant antibodies, particularly chimeric antibodies such as humanised antibodies. Chimeric antibodies are made up of molecular fragments of antibodies of two or more different animal species. Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies which contain minimal polypeptide sequences derived from non-human immunoglobulin. A humanized antibody is primarily a human immunoglobulin (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. Such

modifications may be made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al, Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992).

In a particularly preferred embodiment of the present invention, the composition of monoclonal antibodies that is concentrated by membrane filtration comprises primatized monoclonal antibodies. These are chimeric, humanized antibodies that have the variable region of a non-human primate, e.g., a monkey, and the constant region of a human antibody. Primatized antibodies have little or no immunogenicity in humans. They are described in U.S. Patent No. 6,136,310, and in U.S. Patent No. 5,658,570, the contents of which are incorporated herein in their entirety.

In a preferred embodiment of the present invention, the composition of monoclonal antibodies that is concentrated by membrane filtration comprises monoclonal antibodies selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies. Such antibodies have been described in the scientific literature, and can be prepared by routine methods.

In another preferred embodiment of the present invention, the composition of monoclonal antibodies that is concentrated by membrane filtration comprises monoclonal antibodies selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies. Rituxan, is a chimeric anti-CD20 antibody from IDEC

Pharmaceuticals Corp. and Genentech, Inc., for the treatment of non-Hodgkin's lymphoma, and is described in U.S. Patent No. 6,399,061, the contents of which are incorporated herein in their entirety. IDEC-114 is an anti-CD80 MAb for treating autoimmune diseases and preventing organ transplant rejection that is described in U.S. Patent No. 6,113,898. IDEC-131 is an anti-gp39 MAb that is also useful for treating autoimmune diseases, as described in U.S. Patent No. 6,001,358. IDEC-151 is an anti-CD4 MAb that is useful for T cell depletion therapy, as described in U.S. Patent No. 6,136,310. IDEC-152 is an anti-CD23 antibody that inhibits IL-4-induced IgE production by B cells and is useful for treating IgE-mediated pathologies such as atopic dermatitis, allergic rhinitis, and asthma, as described in U.S. Patent No. 6,011,138. The contents of the U.S. patents describing making and using the foregoing therapeutic MAbs have been incorporated herein in their entirety.

Concentration by Membrane Ultrafiltration

The present invention stems from the discovery that the stability and viscosity of a antibody preparation subjected to concentration by membrane ultrafiltration is sensitive to the type of buffer present in the preparation, and that certain buffers, in particular, histidine and acetate, unexpectedly lower the viscosity of an antibody preparation, reduce antibody aggregation, and increase the rate of concentration of the antibody preparation by membrane filtration, relative to what is achieved using other buffers.

While diverse methods for preparing and purifying therapeutic MAbs have been developed, they typically have in common a final step of concentration by ultrafiltration that precedes formulation of the final product – the pharmaceutical preparation that is to be administered. Ultrafiltration of MAbs is generally carried out by filtering the antibody solution under pressure through a membrane filter with pores that retain polypeptides of

50-200 kilodaltons while allowing smaller molecules to pass through. The efficiency of the ultrafiltration operation can be affected by the viscosity of the solution, the solubility, and amount of aggregates of the protein. Diafiltration is the fractionation process that washes smaller molecules through the membrane and leaves the larger molecules of interest in the retentate.

There are two membrane filtration methods that are commonly used for ultrafiltration. In direct flow filtration (DFF) the feed (the solution to be filtered) is forced directly toward the membrane as shown in Figure 1. As a result, molecules larger than the pores aggregate at the membrane surface and form a gel that blocks the flow of the smaller molecules through the pores, so that the flux rate decreases rapidly as filtration proceeds, as shown in Figure 2. DFF is also called "normal flow filtration" because the fluid flow occurs in a direction normal to the membrane surface. The protein solution is often stirred during DFF in order to keep the retained protein from aggregating and blocking the pores of the membrane. Surprisingly, depending on the conditions (e.g., pressure and flow rates), the shear forces caused by circulating retentate through a TFF system (described below) may cause more aggregation and precipitation that is caused by stirring a protein solution during DFF (see Relton, Example 3, columns 10-11).

The other main ultrafiltration process is tangential flow filtration (TFF), in which the sample flows across the surface of the membrane as pressure on the solution forces smaller molecules in the solution outwards through the pores of the membrane, as shown in Figure 3. The flow of solution across the membrane during TFF helps prevent a gel of aggregated molecules from forming on the surface of the membrane of that blocks the pores and prevents smaller molecules from passing through. As a result, the flux rate for TFF drops off much more slowly as filtration proceeds than occurs during DFF, as shown in Figure 4. For this reason, TFF is the preferred membrane ultrafiltration method for

preparing highly concentrated solutions of MAbs that are useful in formulating pharmaceutical MAb preparations. TFF systems for performing ultrafiltration of MAB solutions are commercially available, for example, from Millipore Corp. (Bedford, MA), Pall Corp. (East Hills, NY), or Marcon Wines and Filters (Oakville, Ontario).

In addition to concentrating, a TFF system can be used to exchange buffers or to reduce the concentration of undesirable species, e.g., to the lower concentration of salt, in the preparation. This is done by introducing fresh buffer while filtering under pressure to remove the original solvent and other small molecules that are not retained by the filter. By concentrating a solution to half its volume and adding new buffer four times, it is possible to remove over 96% of the salt in a preparation. More than 99% of the original buffer in a solution can be replaced by adding up to 7 volumes of new buffer during continuous diafiltration.

Pharmaceutical Formulations

The terms "pharmaceutical formulation" and "pharmaceutical composition" refer to preparations which are in such form as to permit the biological actvity of the active ingredients to be unequivocally effective, and which contain no additional components which are toxic to the subjects to which the formulation would be administered. "Pharmaceutically acceptable" carriers (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

Concentrated antibody preparations prepared according to the present invention may be used to prepare pharmaceutical formulations by combining antibodies of the concentrated antibody preparation produced according to the disclosed invention with one or more pharmaceutically acceptable carriers to produce a pharmaceutical composition.

Such a pharmaceutical composition may optionally be prepared to include one or more additional therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The antibodies and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously. The compositions for parenteral administration will commonly comprise a solution of an antibody or fragment thereof of the invention or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., water, buffered water, 0.4 k saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well-known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody or fragment thereof of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5 k, usually at or at least about 1% to as much as 15 or 20% by weight, and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and 50 mg. of an antibody or fragment thereof of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain 250 ml. of sterile Ringer's solution, and 150 mg. of an antibody or fragment thereof of the invention. Actual methods for preparing parenterally administrable compositions are well-known or will be apparent to those skilled in the art, and are described in more detail in, for example, Remington's

Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa., hereby incorporated by reference herein.

Therapeutic Uses

The present invention provides an improvement to a method of therapy that includes the administration of a pharmaceutical composition comprising an antibody. The improvement comprises administering a pharmaceutical composition that comprises a concentrated antibody preparation comprising histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM, and further comprises a therapeutically effective dose of a therapeutic antibody. As disclosed herein, the concentrated antibody preparation comprising histidine or acetate buffer according to the present invention has reduced viscosity and increased stability relative to other preparations.

The above-described improved method of therapy comprises, for example, administering a therapeutically effective dose of therapeutic antibody to a patient suffering from a disease selected from the group consisting of cancer, allergic disorders, and autoimmune diseases. A particularly valuable embodiment of the invention comprises administering a pharmaceutical composition comprising a therapeutically effective dose of therapeutic antibody to treat lymphoma. Of particular benefit will be administration of therapeutic monoclonal antibodies selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies. The concentrated antibody preparation used for the improved method may comprise a therapeutically effective dose of therapeutic primatized monoclonal antibodies. Treatment of disease by administering therapeutic monoclonal antibodies selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies is also beneficial. The disclosed pharmaceutical composition comprising a concentrated antibody preparation comprising

histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM is administered used in the same manner as the pharmaceutical compositions comprising a therapeutically effective dose of therapeutic antibodies of the prior art.

A "Kit" Containing a Concentrated Preparation of Therapeutic Antibodies

The present invention further provides a kit that is useful for the treatment of a mammal suffering from, or predisposed to, a disorder. Inside the kit is at least one container having a pharmaceutical composition prepared from a concentrated antibody preparation comprising a therapeutically effective dose of a therapeutic antibody, and also comprising histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM, according to the invention. The kit is also labeled with a label or contains an insert that indicating that the pharmaceutical composition within the kit may be used to treat the aforementioned disorder. The kit may be contain a therapeutically effective dose of therapeutic monoclonal or polyclonal antibodies. In a preferred embodiment, the therapeutic antibody is an IgG antibody. In another preferred embodiment, the therapeutic antibody is a monoclonal antibody. In another preferred embodiment, the therapeutic antibody is a primatized monoclonal antibody. In an especially preferred embodiment, the kit contains a therapeutically effective dose of therapeutic antibody that is useful for treating a disorder selected from the group consisting of cancer, allergic disorders, autoimmune diseases, and lymphoma. In a preferred embodiment, the therapeutic antibody is selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies. In a particularly preferred embodiment, the therapeutic antibody is selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies.

EXAMPLE

Tangential flow filtration is one of the most commonly used techniques in the processing steps to concentrate protein and dialfiltrate the material for the final formulation. The success of its operation could significantly influence product yield and stability. Thus it is important to explore the factors that might improve the efficiency of this operation. To this end, in the present study, we examine the effects of buffer species and pH on the performance of tangential flow filtration and their effects on product stability. This example demonstrates that MAb preparations formulated with relatively low concentrations of acetate or histidine buffers (5-40 mM) have lower viscosity and less aggregation than a preparation of the same MAb formulated with citrate buffer.

Tangential flow filtration (TFF) is commonly used for diafiltration and concentration of a MAb preparation in the final steps of preparing an highly concentrated aqueous MAb solution suitable for use as a pharmaceutical formulation. The efficiency of TFF can be affected by the viscosity of the solution, the solubility of the protein, and extent to which the protein has formed aggregates in the solution.

Materials and Methods

Stock solutions of IDEC-114 MAbs at a concentration of 10 mg/ml in 10 mM citrate (pH 6.5) and 150 mM NaCl or in 25 mM sodium acetate (pH 6.0) and 220 mM glycine, stored aseptically at 2-8°C, were obtained. IDEC-114 MAbs are primatized antibodies – chimeric, recombinant IgG1 MAbs that have human constant regions and macaque monkey variable regions that bind CD80. The stock IDEC-114 MAb solutions were concentrated to 25 mg/ml by diafiltration at room temperature, using a LabScale Tangential Flow Filtration (TFF) System equipped with Pellicon XL (PLCTK 30) membrane cassettes (Millipore Corp.,Bedford, MA). Six aqueous solutions consisting essentially of IDEC-114 MAbs at 25 mg/ml and a selected buffer at a desired pH were

en prepared by diafiltration at room temperature by exhanging one volume of antibody buffer for eight volumes of each of the following test buffers: 20 mM sodium acetate, pH 5.5 and 6.0; 20 mM sodium citrate, pH 5.5 and 6.0; and 20 mM histidine/HCl, pH 5.5 and 6.0. The chemicals used to prepare the buffer solutions were: sodium acetate (Sigma, S-1304); sodium citrate (Fisher, S279-500), and histidine (JT Baker, product # 2080.06).

The samples were then further concentrated in the Labscale TFF System until the permeate flow rate approached 1ml/min, at which time the antibody solutions were concentrated to above 150 mg/ml. The time required to achieve a concentration of 150 mg/ml was recorded. To maintain the uniformity of all the operations, the system flow rate was fixed at 80 ml/min, under optimal retention pressure, during the whole process.

Periodically during concentration by TFF, small aliquots of the MAb solutions were withdrawn for determination of protein concentration and measurement of viscosity and turbidity, at which time the permeate flow rate was also recorded. After TFF, samples were removed from the system and passed through an Acrodisc PF Syringe Filter 0.8/0.2 µm Supor membrane (Gelman Laboratory) to remove soluble aggregates.

Effects of buffer species and pH on the operational efficiency of TFF

Protein concentrations were determined by UV spectrophotometric scan over the course of TFF. The samples were accurately diluted to 100X or 200X in water, depending on the concentration, and the absorbance at 280 nm was read with a Shimadzu Multispec-1501 photo diode array spectrophotometer against water as blank. Figure 5 shows the permeate flow rate at different concentrations of antibody during the TFF process, from which it can clearly be seen that the permeate flow rates followed the trend: histidine>acetate>citrate. There was no trend regarding the pH effect on the flow rate. At concentrations above 80 mg/ml the permeate flow rate reduced, and we saw no significant

difference between the flow rates of histidine- and acetate-formulated samples; however, the permeant flow rates for both of these were significantly higher than for the citrate-formulated material at the two pH values tested. The time it took to concentrate 240 ml of 25 mg/ml IDEC-114 to 150 mg/ml is listed on Table I. On average, antibody solutions formulated with citrate took about 30% more time to concentrate than those formulated with acetate, and about 50% more time than those formulated with histidine.

Table I

Formulation	Time (min)
20 mM histidine/HCl, pH 5.5	19.0
20 mM histidine/HCl, pH 6.0	20.4
20 mM sodium acetate, pH 5.5	23.4
20 mM sodium acetate, pH 6.0	20.5
20 mM sodium citrate, pH 5.5	27.7
20 mM sodium citrate, pH 6.0	30.4

Turbidity

During TFF operation, the antibody molecules were continuously pumped through the system for numerous cycles, and so are subjected to strong shearing forces that could potentially result in aggregation and increase the turbidity of the solution. Figure 6 depicts the turbidity profile of the formulated antibody, as measured by OD320 over the course of the concentration process. It is obvious that-citrate formulated MAb solution had much higher turbidity than acetate- and histidine-formulated solutions at both pH values. With the exception of the pH 6.0 acetate formulation, the latter two buffers had very similar profiles. This result indicates that histidine and acetate buffers offer significantly better protection against aggregation of antibody molecules relative to citrate buffer.

Agitation Assay

To further measure the stability of the concentrated MAbs an accelerated aggregation study was performed. 3 ml of each filtered formulation from the TFF process were put into a sterile 5 cc type I glass vial, which was stoppered with a teflon-faced, gray, butyl rubber stopper, and capped with crimp seal. The vials then were put on a shaker set at 700 rpm and agitated at room temperature for 72 hours. Each concentrated antibody formulation was filtered through a 0.2μ membrane and its concentration was adjusted to 150 mg/ml. OD320 and OD 580 readings taken before and after the agitation are shown in Table II. Although the initial OD320 readings were all relatively low and were very similar to each other, after agitation, both citrate-formulated antibodies had the highest turbidity, followed by histidine and acetate. A similar trend is reflected in the OD580 measurements. We did not find any specific pH trend for the turbidity, but within the same buffer, different pH values resulted in different levels of aggregation.

Table II

Formulation	OD_{320}		OD_{580}	
	Before	After	Before	After
20 mM Histidine, pH 5.5	0.292	0.868	0.007	0.135
20 mM Histidine, ph 6.0	0.306	1.010	0.007	0.168
20 mM Acetate, pH 5.5	0.291	0.868	0.011	0.148
20 mM Acetate, pH 6.0	0.282	0.610	0.012	0.082
20 mM Citrate, pH 5.5	0.375	>2.0	0.017	0.470
20mM Citrate, pH 6.0	0.371	1.219	0.013	0.210

Viscosity of the different IDEC-114 formulations

The kinematic viscosities of the samples concentrated to 150 mg/ml were measured by a calibrated size 2 Cross Arm Viscometer (VWR). All the measurements

were done at room temperature (23±3°C) with 3 ml of solution. Figure 7 is a bar graph that shows the measured kinematic viscosity of the 6 different formulations for IDEC-114. As expected, the citrate-formulated solutions had the highest viscosity, followed by the acetate solutions, and the histidine-buffered solutions had the lowest viscosity. Again, within the same buffer species, pH seemed have an effect on the viscosity, however across the buffer species no specific pH trend could be found.

WE CLAIM:

1. A concentrated antibody composition comprising an aqueous solution of antibodies and histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM.

2. The composition of claim 1, wherein the concentration of histidine or acetate buffer is in the range of from 20 mM to 25 mM.

3. The composition of claim 1 that has pH in the range of from 4.5 to 7.0.

4. The composition of claim 1 that has pH in the range of from 5.5 to 6.0.

5. The composition of claim 1, comprising IgG antibodies.

6. The composition of claim 1, wherein the antibodies are monoclonal antibodies.

7. The composition of claim 6, wherein the concentration of monoclonal antibodies is at least 100 mg/ml.

8. The composition of claim 6, comprising primatized monoclonal antibodies.

9. The composition of claim 6, comprising monoclonal antibodies selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies.

10. The composition of claim 6, comprising monoclonal antibodies selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies.

11. A method for producing a concentrated antibody preparation comprising the steps of:

(a) providing an initial antibody preparation comprising histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM; and

(b) subjecting the initial antibody preparation to membrane filtration that removes water and buffer but not antibodies from the antibody preparation,

thereby producing an antibody preparation having a higher concentration of antibodies than the initial antibody preparation.

12. The method of claim 11, wherein the concentration of histidine or acetate buffer is in the range of from 20 mM to 25 mM.

13. The method of claim 11, wherein the pH of the antibody preparation is in the range of from 4.5 to 7.0.

14. The method of claim 11, wherein the pH of the antibody preparation is in the range of from 5.5 to 6.0.

15. The method of claim 11, wherein the antibody preparation comprises IgG antibodies.

16. The method of claim 11, wherein the antibody preparation comprises monoclonal antibodies.

17. The method of claim 16, wherein the concentration of monoclonal antibodies in the antibody preparation is at least 100 mg/ml.

18. The method of claim 16, wherein the antibody preparation comprises primatized monoclonal antibodies.

19. The method of claim 16, wherein the antibody preparation comprises monoclonal antibodies selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies.

20. The method of claim 16, wherein the antibody preparation comprises monoclonal antibodies selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies.

21. An improved method for producing a concentrated antibody preparation comprising the steps of:

(a) providing an initial antibody preparation comprising antibodies and buffer; and

(b) subjecting the initial antibody preparation to membrane filtration that removes water and buffer but not the antibodies from the antibody preparation,

thereby producing an antibody preparation having a higher concentration of antibodies than the initial antibody preparation;

the improvement consisting of using buffer selected from histidine or acetate at a concentration in the range of from 5 mM to 40 mM.

22. The method of claim 21, wherein the concentration of histidine or acetate buffer is in the range of from 20 mM to 25 mM.

23. The method of claim 21, wherein the pH of the antibody solution is in the range of from 4.5 to 7.0.

24. The method of claim 21, wherein the pH of the antibody solution is in the range of from 5.5 to 6.0.

25. The method of claim 21, wherein the antibody preparation comprises IgG antibodies.

26. The method of claim 21, wherein the antibody preparation comprises monoclonal antibodies.

27. The method of claim 26, wherein the concentration of monoclonal antibodies in the antibody preparation is at least 100 mg/ml.

28. The method of claim 26, wherein the antibody preparation comprises primatized monoclonal antibodies.

29. The method of claim 26, wherein the antibody preparation comprises monoclonal antibodies selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies.

30. The method of claim 26, wherein the antibody preparation comprises monoclonal antibodies selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies.

31. A method for producing a pharmaceutical composition comprising antibodies as the active ingredient, comprising the steps of:

(a) providing an initial antibody preparation comprising histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM; and

(b) subjecting the initial antibody preparation to membrane filtration that removes water and buffer but not antibodies from the antibody preparation, thereby producing an antibody preparation having a higher concentration of antibodies than the initial antibody preparation; and

(c) combining antibodies of the concentrated antibody preparation of step (b) with one or more pharmaceutically acceptable carriers to produce a pharmaceutical composition.

32. The method of claim 31, wherein the concentration of histidine or acetate buffer is in the range of from 20 mM to 25 mM.

33. The method of claim 31, wherein the pH of the initial antibody solution is in the range of from 4.5 to 7.0.

34. The method of claim 31, wherein the pH of the initial antibody solution is in the range of from 5.5 to 6.0.

35. The method of claim 31, wherein the antibody preparation comprises IgG antibodies.

36. The method of claim 31, wherein the antibody preparation comprises monoclonal antibodies.

37. The method of claim 36, wherein the concentration of monoclonal antibodies in the antibody preparation is at least 100 mg/ml.

38. The method of claim 36, wherein the antibody preparation comprises primatized monoclonal antibodies.

39. The method of claim 36, wherein the antibody preparation comprises monoclonal antibodies selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies.

40. The method of claim 36, wherein the antibody preparation comprises monoclonal antibodies selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies.

41. An improved method of therapy that includes the administration of a pharmaceutical composition comprising an antibody,

the improvement comprising administering a pharmaceutical composition that comprises a concentrated antibody preparation comprising histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM, and further comprises a therapeutically effective dose of a therapeutic antibody.

42. The improved method of claim 41, wherein the concentrated antibody preparation comprises a therapeutically effective dose of therapeutic IgG antibodies.

43. The improved method of claim 41, wherein the concentrated antibody preparation comprises a therapeutically effective dose of therapeutic monoclonal antibodies.

44. The improved method of claim 43, wherein the concentration of therapeutic monoclonal antibodies in the concentrated antibody preparation is in the range of from 25 to 350 mg/ml prior to being combined with the pharmaceutical composition

45. The improved method of claim 43, wherein the concentration of therapeutic monoclonal antibodies in the concentrated antibody preparation is in the range of from 50 to 250 mg/ml prior to being combined with the pharmaceutical composition.

46. The improved method of claim 43, wherein the concentrated antibody preparation comprises a therapeutically effective dose of therapeutic primatized monoclonal antibodies.

47. The improved method of claim 43, wherein the concentrated antibody preparation comprises a therapeutically effective dose of therapeutic monoclonal antibodies selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies.

48. The improved method of claim 43, wherein the concentrated antibody preparation comprises a therapeutically effective dose of therapeutic monoclonal antibodies selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies.

49. The improved method of therapy of claim 41, comprising administering a pharmaceutical composition comprising a concentrated antibody preparation that comprises a therapeutically effective dose of therapeutic antibody to elicit a therapeutic response in a patient in need of such treatment.

50. The improved method of therapy of claim 49, comprising administering a therapeutically effective dose of therapeutic antibody to a patient suffering from a disease selected from the group consisting of cancer, allergic disorders, autoimmune diseases, and lymphoma.

51. A kit useful for the treatment of a mammal suffering from or predisposed to a disorder comprising at least one container having a pharmaceutical composition that comprises a concentrated antibody preparation comprising histidine or acetate buffer at a concentration in the range of from 5 mM to 40 mM, and further comprises a

therapeutically effective dose of a therapeutic antibody deposited therein and a label or an insert indicating that said pharmaceutical composition may be used to treat said disorder.

52. The kit of claim 51, wherein said therapeutic antibody is an IgG antibody..

53. The kit of claim 51, wherein said therapeutic antibody is a monoclonal antibody.

54. The kit of claim 53, wherein the concentration of said therapeutic antibody in said concentrated antibody preparation is in the range of from 25 to 350 mg/ml prior to being combined with the pharmaceutical composition

55. The kit of claim 53, wherein the concentration of said therapeutic antibody in said concentrated antibody preparation is in the range of from 50 to 250 mg/ml prior to being combined with the pharmaceutical composition.

56. The kit of claim 53, wherein said therapeutic antibody is a primatized monoclonal antibody.

57. The kit of claim 53, wherein said therapeutic antibody is selected from the group consisting of anti-CD80, anti-gp39, anti-CD4, anti-CD23, and anti-CD20 antibodies.

58. The kit of claim 53, wherein said therapeutic antibody is selected from the group consisting of Rituxan, IDEC-114, IDEC-131, IDEC-151, and IDEC-152 antibodies.

59. The kit of claim 51, that is useful for the treatment of a mammal suffering from or predisposed to a disorder selected from the group consisting of cancer, allergic disorders, autoimmune diseases, and lymphoma.

ABSTRACT

The present invention provides a method for producing a concentrated antibody preparation that includes the steps of: (a) obtaining an initial antibody preparation that is an aqueous solution of antibodies and histidine or acetate buffer at a concentration in the range of from 5 to 40 mM; and (b) subjecting the antibody preparation to membrane filtration so as to remove water and buffer but not antibodies from the antibody preparation, thereby producing an antibody preparation having a higher concentration of antibodies than the initial antibody preparation. The concentrated antibody preparations produced by the method are less viscous and more stable than those of other formulations. The invention further includes concentrated antibody preparations produced by the method, pharmaceutical compositions made using such preparations, and therapeutic methods in which such pharmaceutical compositions are administered to treat diseases.

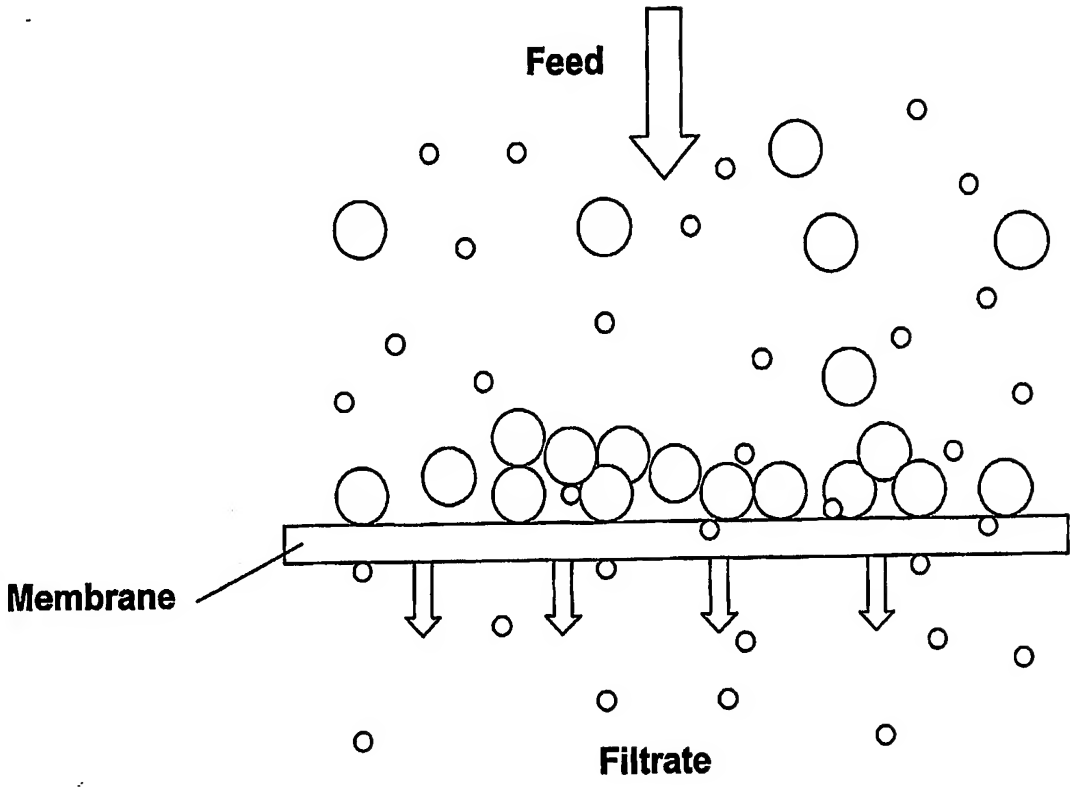

Figure 1

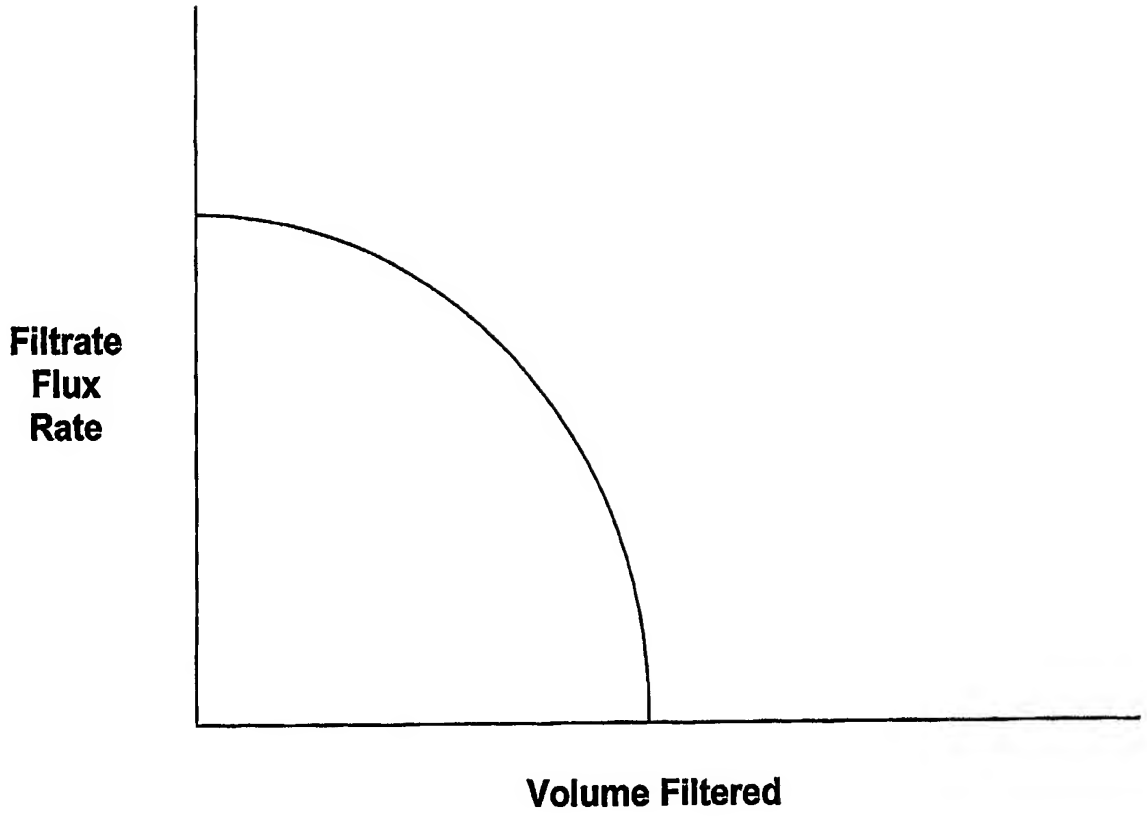

Figure 2

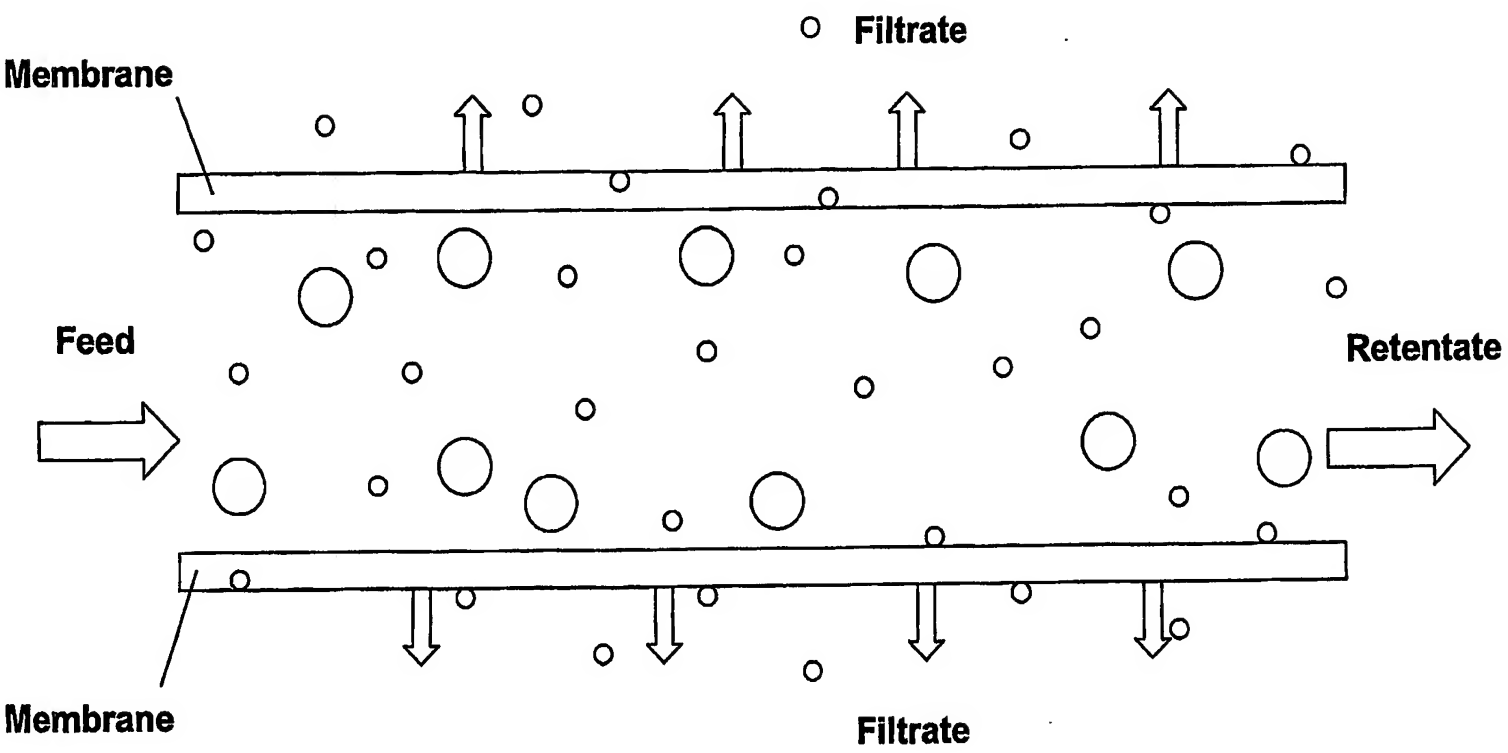

Figure 3

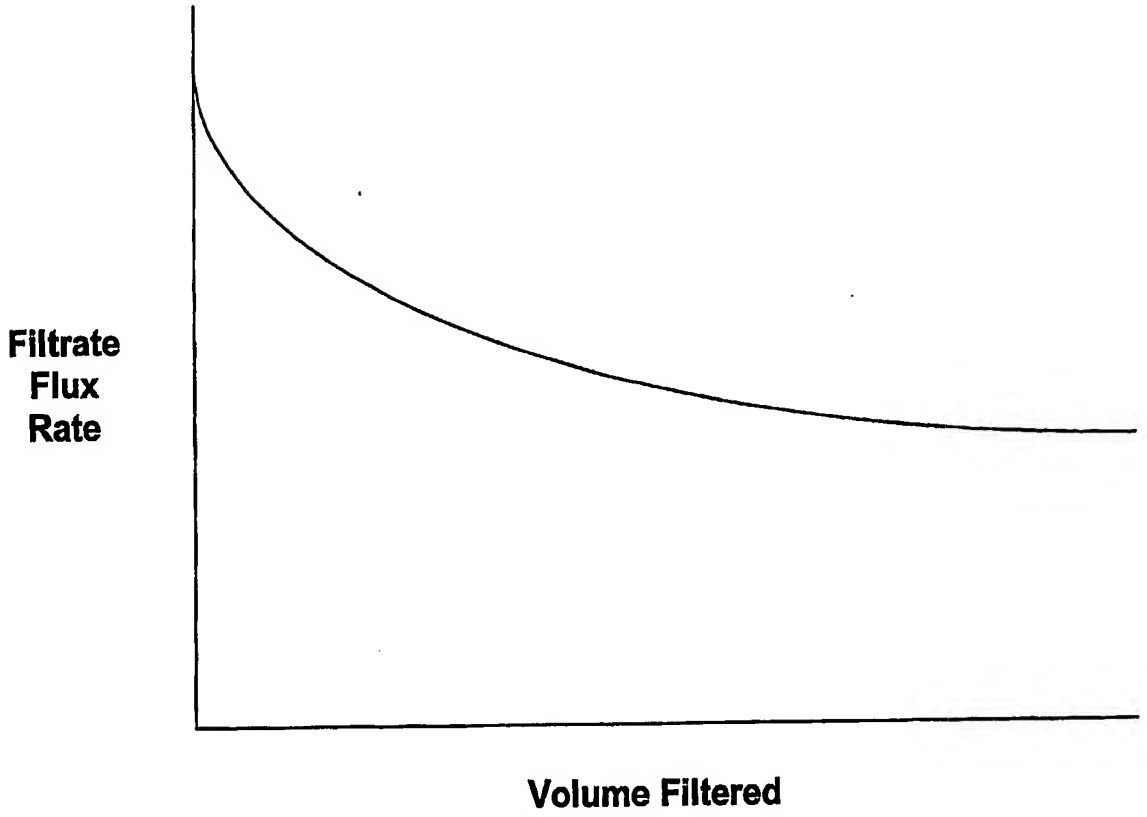

Figure 4

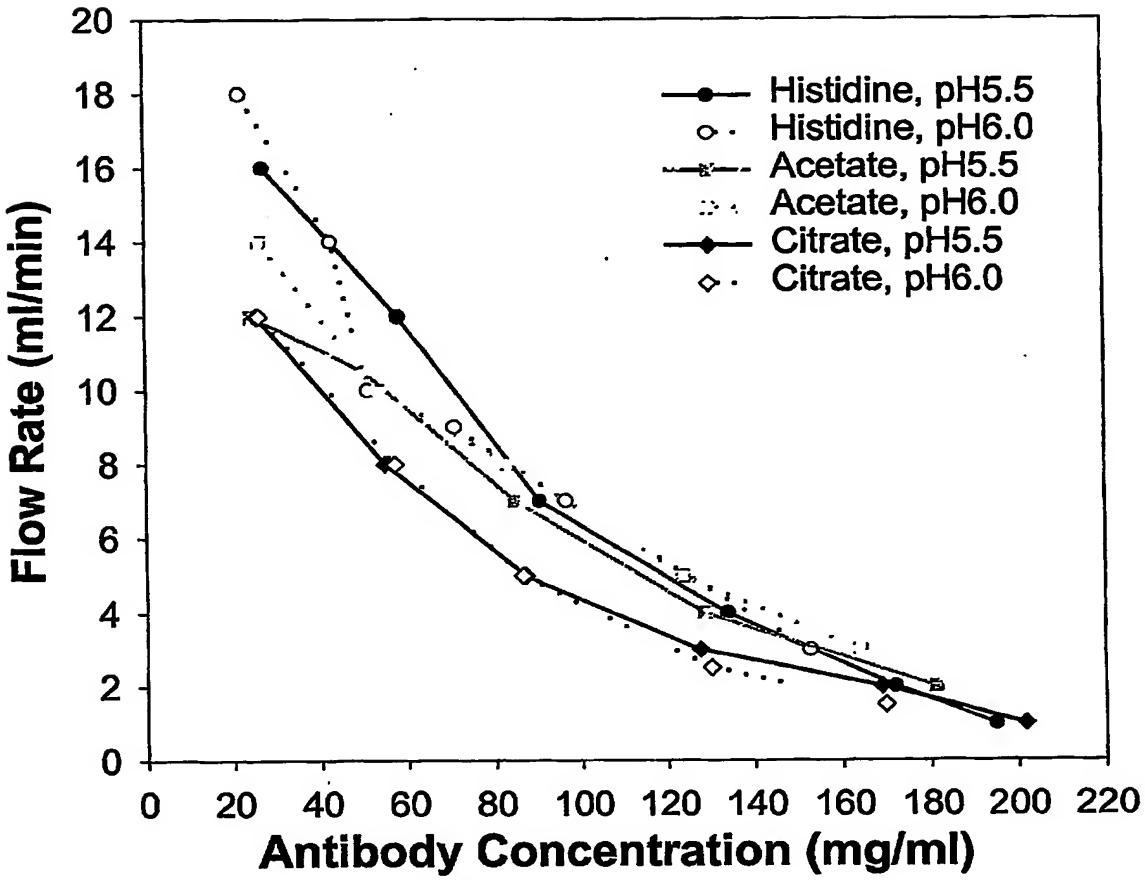
Flow Rate (ml/min)
Antibody Concentration (mg/ml)
Histidine, pH5.5
Histidine, pH6.0
Acetate, pH5.5
Acetate, pH6.0
Citrate, pH5.5
Citrate, pH6.0
0
2
4
6
8
10
12
14
16
18
20
0
20
40
60
80
100
120
140
160
180
200
220

Figure 5

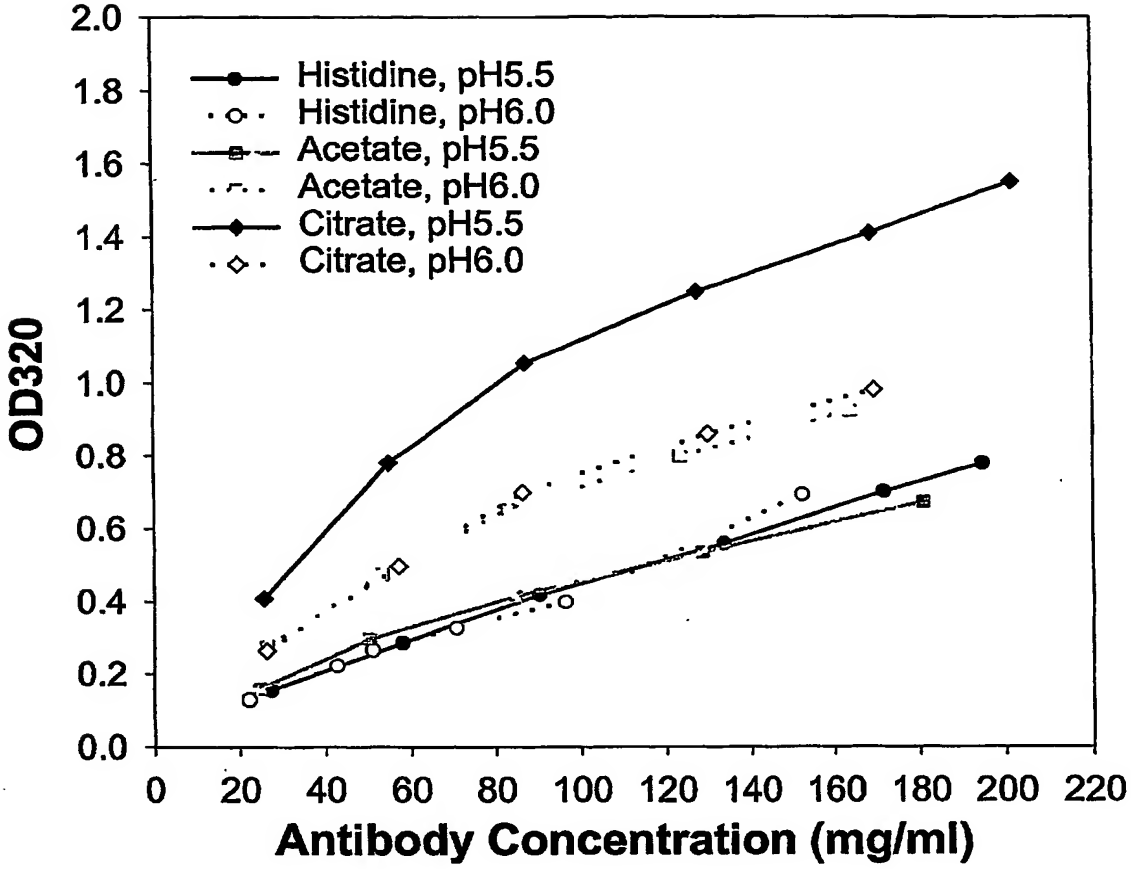
Histidine, pH5.5
Histidine, pH6.0
Acetate, pH5.5
Acetate, pH6.0
Citrate, pH5.5
Citrate, pH6.0
OD320
Antibody Concentration (mg/ml)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
0
20
40
60
80
100
120
140
160
180
200
220

Figure 6

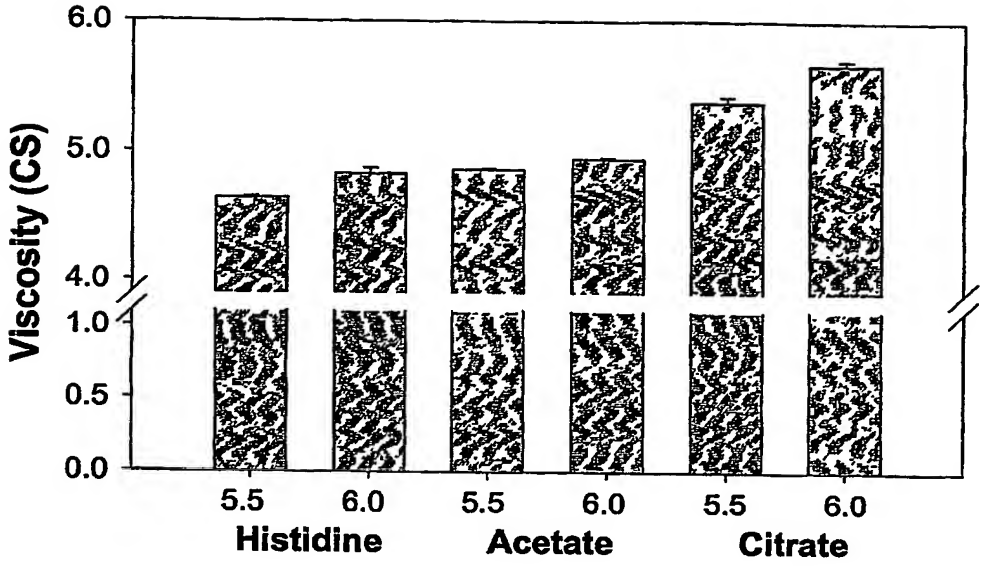
6.0
5.0
4.0
1.0
0.5
0.0
Viscosity (CS)
5.5
6.0
5.5
6.0
5.5
6.0
Histidine
Acetate
Citrate

Figure 7